Atrial Natriuretic Peptide Increases Urinary Albumin Excretion in Men with Type 1 Diabetes Mellitus and Established Microalbuminuria

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Raised plasma concentrations of atrial natriuretic peptide (ANP) have been reported in patients with Type 1 (insulin dependent) diabetes mellitus (DM) who have poor glycaemic control and are associated with the presence of microalbuminuria. To test the hypothesis that elevations in plasma ANP concentration increase urinary albumin excretion in Type 1 DM, we have studied the effects of intravenous infusions of ANP in eight such subjects with established microalbuminuria. Blood glucose was maintained between 4 and 7 mmol l^{-1} in all subjects for the duration of studies; after euglycaemia had been established, a standard oral water load (20 ml kg^{-1} plus replacement of urinary losses) was given. Once steady state diuresis was attained, subjects received intravenous infusion of either placebo (0.9 % saline), low dose (2.5 pmol kg^{-1} min^{-1}) or high dose (5.0 pmol kg^{-1} $kg\ min^{-1}$) ANP solution in a randomized, double-blind protocol. Infusion of ANP caused a dose-dependent increase in urinary albumin excretion rate (placebo, 11.3 (SD 8.9) to 8.7 (SD 6.8) $\mu g\ min^{-1}$; low dose ANP, 12.4 (SD 9.9) to 26.5 (SD 27.5) $\mu g\ min^{-1}$, $p < 0.01$; high dose ANP 10.3 (SD 7.3) to 36.6 (SD 28.5) $\mu g\ min^{-1}$, $p < 0.001$, ANOVA). Only high dose ANP caused an increase in urine flow. Blood glucose remained unchanged in all studies. We conclude that intravenous infusions of ANP cause a dose-dependent increase in urinary albumin excretion rate in Type 1 DM subjects with microalbuminuria. These data support the hypothesis that ANP has albuminuric actions which may contribute to microalbuminuria in Type 1 DM. © 1998 John Wiley & Sons, Ltd.

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Introduction

Plasma concentrations of atrial natriuretic peptide (ANP) have been found to be elevated in some patients with Type 1 (insulin dependent) diabetes mellitus (DM),[1–7] though normal plasma ANP concentrations have been reported elsewhere.[8] Raised plasma ANP concentrations have been reported particularly in association with microalbuminuria,[1,3,4] poor glycaemic control[1] and high blood pressure.[5] The association in Type 1 DM of elevated plasma ANP concentrations and the presence of microalbuminuria is potentially of clinical importance, as ANP has been reported to induce microalbuminuria in healthy volunteers[9] and to increase the rate of urinary protein excretion in patients with nephrotic syndrome[10] and chronic glomerulonephritis.[11] This raises the possibility that elevated plasma concentrations of ANP, such as those reported in Type 1 DM patients with poor glycaemic control,[1] may have actions to increase urinary albumin excretion.

There are published data in Type 2 (non-insulin dependent) diabetes mellitus which suggest that plasma ANP concentrations increase in the early phase of microalbuminuria and that this increase is related to the development of microalbuminuria[12] but there is little information on the effects of moderate increases in plasma ANP in Type 1 disease. There have been reports which show that bolus intravenous injections of pharmacological doses of ANP have marked microalbuminuric effects in Type 1 DM,[13,14] and recently it was shown that sustained intravenous infusions of ANP, which produced pharmacological plasma ANP concentrations, increased urinary albumin excretion in such patients.[15] However, although there is this evidence that pharmacologically elevated plasma ANP concentrations can increase urinary albumin excretion in human Type 1

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Table 1. Results of urine flow rate (ml min^{-1}) prior to water loading, and in the three 15-min periods of steady state diuresis immediately prior to ANP/placebo infusion

	Pre-water load	−30 min	−15 min	Pre-infusion
Placebo	2.1 (1.1)	15.4 (3.4)	16.3 (4.2)	15.6 (4.0)
Low dose ANP	2.9 (1.2)	17.2 (6.0)	17.0 (5.6)	17.6 (6.8)
High dose ANP	2.5 (1.4)	17.1 (5.7)	16.0 (5.0)	17.3 (5.6)

DM, there is little evidence to suggest that moderate elevations in plasma ANP concentrations comparable to those reported in poor glycaemic control[1] or hypertension[5] could increase urinary albumin excretion rate, and there are no data available on whether the magnitude of the increase in albumin excretion rate is dependent on the plasma concentrations of ANP.

In this study we wished to test the hypothesis that intravenous infusions of ANP, designed to induce elevations of plasma ANP concentrations to levels similar to those reported in poor glycaemic control, would result in increased urinary albumin excretion rate in patients with Type 1 DM and established microalbuminuria, and to establish whether the albuminuric effect of ANP is dose dependent.

Methods

Study Population

Eight male volunteers (mean age 27.1 years, range 19 to 35 years)with Type 1 DM (mean duration of diabetes 12 years, range 3 to 19 years), were recruited from the local diabetes clinic. The mean HbA_{1c} was 8.3 % (range 6.2 to 9.8 %); the local non-diabetic laboratory reference range is 3–5.2 %. All patients had established microalbuminuria based on outpatient overnight urinary albumin concentrations of >20 mg l^{-1} on at least three consecutive specimens; the mean concentration was 68.6 mg l^{-1} (range 28 to 186 mg l^{-1}). Three patients had background retinopathy and two were hypertensive, as defined by sitting blood pressure $>$ 140/90 mm Hg; both patients were treated with angiotensin-converting enzyme inhibitors. The study protocol was approved by the Local Research and Ethical Committee and written informed consent was obtained from each subject.

Study Design

The experimental design was a randomized, double-blind study of the effects of infusions of placebo (0.9 % sodium chloride solution), low dose ANP (2.5 pmol kg^{-1} min^{-1}) and high dose ANP (5.0 pmol kg^{-1} min^{-1}) on urinary albumin excretion rate. The doses of ANP infusion were selected to reproduce plasma concentrations of ANP similar to those seen with our in-house radioimmunoassay in hypertension (low dose) and cardiac failure (high dose). Volunteers were studied on three separate occasions, at least 1 week apart; the order of the infusions was randomized. Antihypertensive therapy was withheld for 2 weeks prior to the first study day and was not reinstituted until the full study protocol was completed.

On the days of study, volunteers reported to the research centre after an overnight fast, having withheld their usual morning subcutaneous insulin. They were rested recumbent and intravenous cannulae were inserted into the antecubital veins of both arms, using intradermal lignocaine 1 % as local anaesthesia; one cannula was used for intravneous infusion of insulin and ANP/placebo, and the other for withdrawal of blood.

An intravenous infusion of soluble insulin (Human actrapid,: Novo Nordisk) was then started; blood glucose was measured every 15 min using a BM glucometer (Ames), and on the basis of these measurements, the rate of insulin infusion was adjusted to maintain blood glucose concentrations between 4 and 7 mmol l^{-1}. Once stable euglycaemia had been maintained for 30 min, volunteers voided urine, and drank a standard oral water load of 20 ml kg^{-1}. Volunteers then stood to void urine at 15 min intervals; the volume of urine voided was measured and an equal volume of water given to drink. Water loading was continued until volunteers were in steady state diuresis, as defined by stable urine volumes on three consecutive samples.

Once steady state diuresis was achieved, an intravenous infusion of placebo, low dose ANP (h-ANP, Clinalfa) or high dose ANP was started and continued for 30 min. After discontinuation of ANP infusion observations were continued over a 1 h recovery period.

Blood samples for measurement of ANP were withdrawn just prior to ANP infusion and at the end of the infusion period, transferred into chilled EDTA tubes, centrifuged at 4 °C for 10 min, and plasma separated and stored at −80 °C. Sampling was repeated at the end of ANP infusion. Urine volumes were measured at 15 min intervals during the infusion period and for 1 h after the infusion had stopped; aliquots were removed for later measurement of microalbuminuria and urinary sodium concentration.

Analytical Methods

ANP was extracted from plasma using C18-Sep columns, a technique giving 80 % recovery (unpublished data).

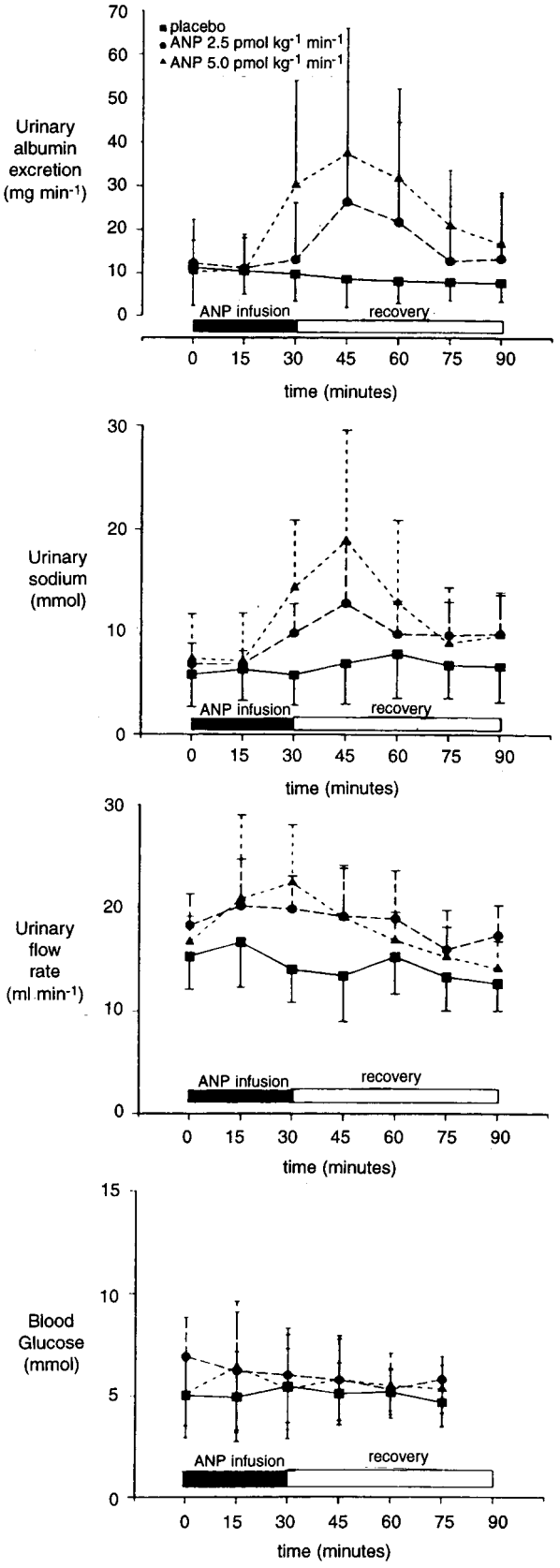

Figure 1. Changes in urinary albumin, urinary sodium, urine flow rate and blood glucose concentration during placebo (■——■), low dose ANP infusion (●--●) and high doseANP infusion (▲---▲)

Plasma ANP concentrations were then measured by an in-house radioimmunoassay using a commercial antibody (Peninsula Laboratories, Belmont, California). The limit of detection of the assay is 2.0 pmol l^{-1} and the intra-assay coefficient of variation is 12.6 %. Urinary albumin concentration was measured by radioimmunoassay using a commercial antibody (Pharmacia, Uppsala, Sweden), with a detection limit of 0.4 mg l^{-1}. Plasma and urinary sodium were measured by flame photometry.

Statistical Analyses

All urinary excretory parameters were analysed using repeated measures analysis of variance (ANOVA). The effects of treatment (placebo or ANP) on all urinary parameters were examined by multifactor ANOVA and the Duncan multiple range analysis. All statistical analysis were performed with Statgraphics Software version 4.0 (Rockville, Maryland, USA).

Results

Water loading significantly increased urine flow rate on all three study days (Table 1). The mean time from start of water loading to steady state diuresis and the start of ANP infusion was 81.9 (SD 23.4) min; there was no difference in the time taken to steady state diuresis between study days. The baseline blood pressure and heart rate readings, steady state urine volumes, urinary sodium, and microalbumin concentration were not significantly different between the three study days (Table 2 and Figure 1). Baseline plasma ANP concentrations were also not different on the three study days (Table 2).

Blood pressure and heart rate remained unchanged during water loading and during subsequent intravenous infusions of ANP/placebo on each of the three study days; there were no differences in blood pressure or heart rate between the study days. Blood glucose concentrations were maintained in the euglycemic range for the duration of the experimental protocol on each of the study days (Figure 1).

Plasma concentrations of ANP rose significantly after both low and high dose ANP infusion (Table 2). Plasma ANP levels were significantly greater after high dose than low dose infusion (37.5 (SD 16.9) vs 25.8 (SD 7.9) pmol l^{-1}, $p < 0.01$, Table 2). Both low and high dose ANP infusion caused an increase in sodium excretion compared to placebo ($p < 0.001$, Figure 1), with the increase in urinary sodium excretion greatest after the high dose infusion ($p < 0.01$). Only high dose ANP infusion caused a significant increase in urine flow rate ($p < 0.001$); there was no change in urine flow rate after either placebo or low dose ANP infusion. Albumin excretion rate remained unchanged following water loading on each of the study days and did not change during placebo infusion (Figure 1). In contrast, both low and high dose ANP infusion caused a significant increase

Table 2. Blood pressure, heart rate, and atrial natriuretic peptide (ANP) concentrations during placebo, low dose and high dose ANP concentrations. Figures given as mean (SD)

	0 min (baseline)	30 min during ANP infusion
Blood pressure (mm Hg)		
placebo	132/82 (10/7)	140/81 (11/7)
ANP 2.5 pmol kg^{-1} min^{-1}	131/79 (12/8)	134/78 (12/7)
ANP 5.0 pmol kg^{-1} min^{-1}	135/81 (6/9)	132/77 (8/7)
Heart rate (beats/min)		
placebo	61 (4.3)	63 (4.6)
ANP 2.5 pmol kg^{-1} min^{-1}	62 (8)	59 (6)
ANP 5.0 pmol kg^{-1} min^{-1}	63 (5)	62 (6)
ANP (pmol l^{-1})		
placebo	5.9 (1.8)	4.2 (1.1)
ANP 2.5 pmol kg^{-1} min^{-1}	5.9 (1.9)	25.8 (7.9)
ANP 5.0 pmol kg^{-1} min^{-1}	5.5 (1.6)	37.5 (16.9)

in urinary albumin excretion compared to placebo ($p < 0.001$). High dose ANP infusion caused a greater increase in urinary albumin excretion than low dose ($p < 0.01$).

Although the maximal urine flow rate occurred at the end of high dose ANP infusion period, urinary sodium excretion and urinary albumin excretion peaked 15 min after cessation of ANP infusion, at a time when urine flow rate was declining (Figure 1). Urine flow rate returned to pre-infusion levels 30 min after cessation of ANP infusion, whereas urinary albumin concentrations was still above baseline values 60 min after ANP infusion was discontinued.

Discussion

The results of this study show that the intravenous infusion of ANP causes a dose-dependent increase in the rate of urinary albumin excretion in Type 1 DM patients with established microalbuminuria. Zietse and colleagues showed that urinary albumin excretion increases in Type 1 DM patients during the intravenous infusion of ANP to raise plasma ANP concentrations to 300–500 pmol l^{-1},[15] whereas the results of this study show that ANP increases urinary albumin excretion at plasma concentrations of the hormone as low as 25 pmol l^{-1}. These lower levels are similar to plasma concentrations reported in the literature to be associated with acute elevations in blood glucose concentration,[16] or chronically poor glycaemic control.[1] Although different radioimmunoassays for ANP were used in these studies, our data suggest that moderate elevations in plasma ANP concentrations, similar to those seen in Type 1 diabetic patients with poor glycaemic control or hypertension, may have actions which contribute to the development of microalbuminuria.

The hypothesis that ANP may have albuminuric actions is supported by animal data, suggesting that ANP may have a pathophysiological role in the development of microalbuminuria in the rat. When rats with streptozotocin-induced diabetes became hyperglycaemic, plasma ANP concentrations rose,[17] and were associated with the development of glomerular hyperfiltration.[18–21] This glomerular hyperfiltration was reversed by treatment with either a specific ANP-antiserum[18] or an ANP receptor antagonist.[19–20] In addition ANP caused an increased in both glomerular filtration rate and urinary albumin excretion; administration of an ANP receptor antagonist produced a significant reduction in both parameters.[21] The results of these studies strongly suggest a significant role for ANP in the development of glomerular hyperfiltration and microalbuminuria in animal models of diabetes, although other factors, such as kinins,[22] and nitric oxide[23] are also important.

The mechanism by which ANP increases urinary albumin excretion in man is not fully understood. Animal data suggest that ANP increases glomerular filtration rate,[17–20] while studies in diabetic humans indicate that ANP alters the size-selectivity of the filtration barrier and increases transcapillary escape of albumin.[15] It is unlikely that increased urinary albumin excretion is simply a reflection of the increased urinary flow associated with ANP infusion; Viberti *et al.*[24] have reported a small, transient increase in urinary albumin excretion rate after water loading in healthy man, but albumin excretion is not affected by water loading in Type 1 DM patients with established microalbuminuria.[25] Certainly water loading had no effect on albumin excretion rate in our present study. Further studies are required to define the mechanism by which ANP exerts its albuminuric effect in human Type 1 DM.

There are data to suggest that ANP causes a more pronounced albuminuric effect in Type 1 DM patients than in non-diabetic controls.[15] A previous study from our laboratory has also shown lower increases in urinary albumin excretion rate in non-diabetic man, than those

achieved in the present study in microalbuminuric diabetic subjects despite higher ANP infusion rates and plasma ANP concentrations.[9]

In summary, we have confirmed that ANP has an albuminuric effect in Type 1 DM patients with established microalbuminuria, and shown that this effect occurs at much lower plasma ANP concentrations than previously reported in the literature. We have also demonstrated for the first time that the albuminuric effect of ANP is dose-dependent, albumin excretion rate increasing as plasma ANP concentrations rise. Further studies are required to investigate the potential role of the albuminuric effects of ANP in the development and progression of microalbuminuria in Type 1 DM but our present data indicate that these are likely to be productive.

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